## REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and the following commentary.

### *I. Introduction*

Upon entry of the above amendment, claims 1-3 and 5-14 are pending in the instant application. Applicants acknowledge withdrawal of the previous rejections under 35 U.S.C. §112, first and second paragraphs and §103(a). Applicants have revised claim 1 to incorporate the step for removing DNA fragments and hairpin loop adapters which have not participated in the ligation reaction by using an exonuclease. Applicants have added claims 13 and 14 to define the series of hairpin loop adapters more specifically. Support for the new claims can be found in claim 2.

### *II. Rejection under 35 U.S.C. §103(a)*

The examiner has rejected claims 1, 3, and 5-12 over U.S. patent No. 5,994,068 to Guilfoyle *et al.* ("Guilfoyle") in view of U.S. Patent No. 5,516,663 to Beckman *et al.* ("Beckman") Applicants respectfully traverse this rejection.

In rejecting these claims, the examiner alleges that "it would have been *prima facie* obvious to one skilled in the art to combine the method of selectively amplifying any fragment generated by a class II restriction enzyme, including adapters specific to fragment ends as taught by Guilfoyle, with the correction of ligated products as taught by Beckman, which is applicable to eliminate hairpin loop structures." Applicants submit, however, that the examiner has overlooked or misapprehended important differences separating the claimed methodology from the method of Guilfoyle and Beckman.

Guilfoyle discloses an indexing system that relies upon fragments not generated by Class IIS or IP enzymes, which offers improved amplification specificity. It was known before Guilfoyle that DNA fragments cleaved by Class IIS or IP enzymes were amplified by annealing PCR primer sites, and nucleic indexing linkers that contain

protruding single strands complementary to the cohesive ends of Class IIS or IP cleavage sites, and ligating these to the DNA fragments. However, the base composition and sequence of the overhang created via cleavage with Class IIS or IP enzymes cannot be predicted. Thus, it is desired to use a Class II enzyme that generates predictable, identical sticky ends on each restriction fragment.

The indexing system of Guilfoyle uses oligonucleotide adaptors for directing PCR amplification that can selectively hybridize to "fragment indexing sequences" of one or more bases immediately adjacent to recognition sites of a Class II restriction enzyme at the termini of a nucleic acid fragment. According to Guilfoyle, each of the oligonucleotide adaptors comprises a single-strand portion and a "duplex portion" that comprises an invading strand and a complementary PCR primer strand hybridized to the invading strand. Guilfoyle further discloses a method for amplifying a restriction fragment using such an oligonucleotide adaptor.

In view of the reasoning of the rejection, the examiner appears to understand that the amplifying method disclosed in Guilfoyle comprises all the steps of the claimed method, except for the step for eliminating a hairpin loop structure by using an alkaline solution, an RNase or a single strand specific exonuclease. Contrary to the examiner's understanding, however, Guilfoyle teaches neither (1) using a hairpin loop structure adaptor nor (2) removing DNA fragments and hairpin adaptors which have not participated in the ligation reaction by using an exonuclease.

In this regard, the examiner seems to equate a universal mismatch base analog, such as the abasic 3-nitropyrrole, to a hairpin loop structure adaptor used in the claimed method. There is no mention of the hairpin loop structure throughout Guilfoyle, however, nor any statement indicating that the universal mismatch base analogue is somehow related to the hairpin loop structure of the adaptor. Rather, Guilfoyle discloses the effect of incorporating a universal mismatch base analogue within the restriction site, in the context of eliciting an effect on the indexing sequence moiety and an effect on the ligase activity. Absent further teachings, therefore, one of ordinary skill in the art would not consider the above description of Guilfoyle constitutes the teaching of the use of a hairpin loop structure adaptor in a method for amplifying DNA fragments.

Furthermore, Guilfoyle does not disclose the use of a step for removing both of the excessive DNA fragments and of adaptors by using exonuclease. In the claimed method, removing the excessive DNA fragments and adaptors is significant in increasing the yield of DNA amplification. However, exonuclease used in the removal step can also affect ligated products of DNA fragments and adaptors. Thus, the claimed method uses an adaptor in a hairpin loop structure to protect the ligated products from the exonuclease, and to selectively remove the excessive DNA fragments and adaptors. Because the Guilfoyle's method does not use an adaptor in a hairpin loop structure, it is not surprising that Guilfoyle does not disclose the step for removing the excessive DNA fragments and adaptors.

In this regard, the most relevant disclosure found in Guilfoyle is that, "after annealing, unbound restriction fragments can be washed away." (See column 10, lines 43-44.) Yet, Guilfoyle does not teach or suggest either the removal of the excessive adaptors or the use of exonuclease for the removal. In fact, "washing" is the only method disclosed in Guilfoyle as a means for removing only "unbound restriction fragments" only. In addition, the description cited by the examiner simply refers to "clean-up, elute in water," which neither implicates the use of exonuclease nor specifies the materials to be cleaned. Such a vague and limited description of Guilfoyle would not have led one of ordinary skill in the art to use a step for removing both DNA fragments and adaptors which have not ligated by using exonuclease to obtain the claimed method. In conclusion, Guilfoyle does not teach or suggest all the claim limitations.

Beckman does not cure such deficiencies of Guilfoyle. The teachings of Beckman relate to a method of improving the Ligase Chain Reaction (LCR). Unlike the PCR, the LCR uses two pairs of probes that hybridize to the target strand for amplification. However, the probes also can be ligated to each other, independent of the presence of the target strand, which results in causing undesirably high background signals in amplified assays. Thus, Beckman provides a method to prevent or reduce such a target independent, spurious signal development. The resultant modified probe and is later corrected in a target-dependent manner, to enable amplification. Exemplary modifications include chemical blockage of reactant groups, or an abasic site and the addition of one or more nucleic acid bases to form an "overhang." The incorporation

of an abasic residue immediately 3′ to the point of intended ligation is one of the modifications suggested in Backman.

The LCR using two pairs of probes that hybridize to the target strand is a clearly distinguished amplification method from the PCR that requires a primer element ligating to the restriction site of DNA fragments, as in the claimed method and in the method of Guilfoyle. That is, the LCR method of Beckman does not disclose the use of an adaptor as a means for introducing a primer, let alone a hairpin loop structure adaptor.

As in Guilfoyle, moreover, there is no teaching or indication in Beckman that an abasic residue is in the form of a hairpin loop structure or how the former is related to the latter. Therefore, cleaving of a modified probe, *e.g.,* ribonucleotide, on the abasic site using, for example, RNase is not equated with opening a hairpin loop structure in the claimed method. Furthermore, Beckman does not mention the removal of both of the excessive DNA fragments and probes.

Claims 5 and 6 are further distinguished from the combined teachings of the prior art in that they are directed to a method using a type IIs or IIip restriction enzyme while the method of Guilfoyle is limited to the use of a Class II restriction enzyme.

To combine the references properly, there must be some teachings or suggestion in the prior art. *In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). Thus, the mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680 (Fed. Cir. 1990). In view of the differences between the LCR and PCR as discussed above, therefore, no reasonable combination of the cited references evidences a motivation somehow to adapt methodology disclosed in Guilfoyle and Beckman to use a hairpin loop structure adaptor, to remove both of the excessive DNA fragments and adaptors and to open the hairpin loop structure.

Even if there were suggestion for such a motivation, the combined teachings of the cited publications would not have led that the skilled artisan to the claimed method since the prior art, alone or in combination, does not provide the slightest teaching or implication of the adaptors in the structure of a hairpin loop, nor removing both of the

excessive DNA fragments and adaptors by using exonuclease followed by eliminating the hairpin loop structure. Accordingly, there is no *prima facie* case of obviousness.

Applicants respectfully request, therefore, that the Examiner withdraw the pending obviousness rejection. Applicants also believe that the present application is in a condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The examiner is invited to contact the undersigned by telephone, if he feels that a telephone interview could advance prosecution.

Respectfully submitted,

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## MARKED UP VERSION SHOWING CHANGES MADE

Below are the marked up amended claims:

1. (Twice Amended) A process for preparing a library of DNA fragments of which terminal sequences are known by using a DNA of which base sequence is completely unidentified, which comprises:

i) digesting a DNA into fragments which have single-strand cohesive ends by using a restriction enzyme,

ii) preparing a series of hairpin loop adapters which have single-strand cohesive ends of which base sequence is known;

iii) ligating the DNA fragments with the hairpin loop adapters prepared in the above step ii) by using a DNA ligase; [and]

iv) removing DNA fragments and hairpin loop adapters which have not participated in the ligation reaction by using an exonuclease; and

[iv)] (v) eliminating a hair pin loop structure only from the DNA fragments which contain the hairpin loop adapters, obtained in step iii), by using an alkaline solution, an RNase or a single strand specific exonuclease.